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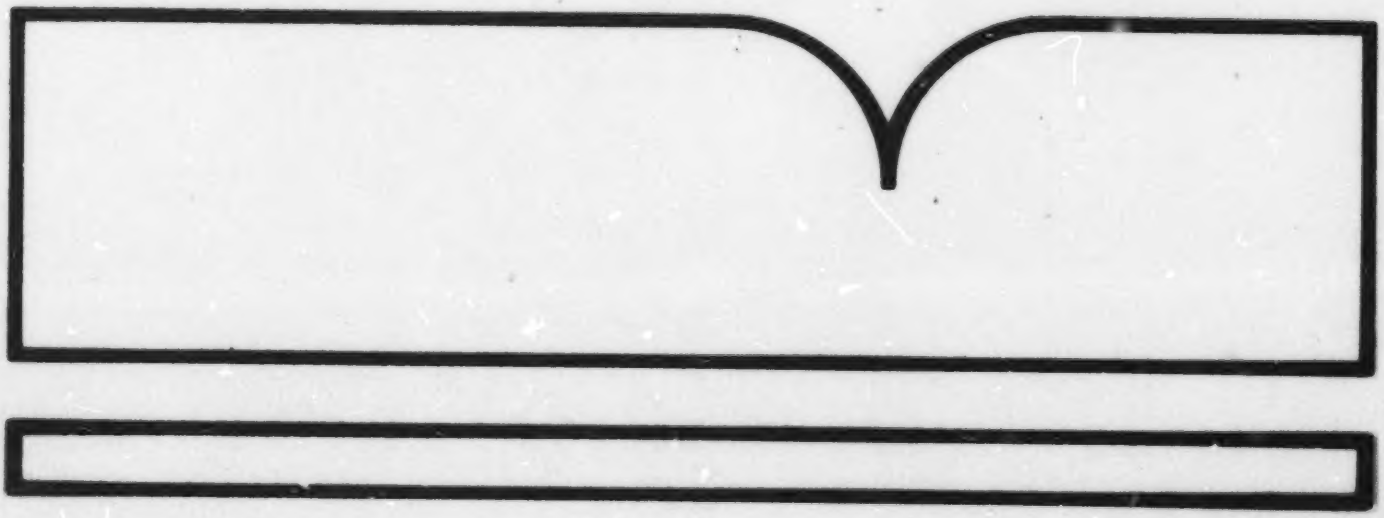

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Acute Toxicity of Triphenyltin Hydroxide to Three Cladoceran Species

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ABSTRACT

The toxicity of an organotin pesticide, triphenyltin hydroxide, was assessed with several freshwater cladoceran species. Daphnia pulex, Daphnia magna, *and* Ceriodaphnia dubia *were exposed for 48 h to triphenyltin hydroxide in static acute toxicity tests. Values of the 48-h trimmed Spearman–Karber* EC_{50}*s for the three species were found to be 14·5, 16·5, and 11·3 µg litre*$^{-1}$*, respectively. Analysis of variance performed on* EC_{50} *values of replicates revealed no significant differences between the three species. Methods were employed which decrease animal handling stress and increase the accuracy and precision of the concentrations.*

INTRODUCTION

Triphenyltin hydroxide (TPTH) is registered as an organotin fungicide. Common names for TPTH include fentin hydroxide and Du-Ter. It is used to control fungus diseases on potatoes, sugar beets, peanuts, pecans, carrots, soybeans, cocoa, and coffee, although its most direct access to the aquatic environment is through its use in rice paddies (Bock, 1981; *Farm Chemicals*

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Handbook, 1987). Some information is available on the toxicity of TPTH to aquatic organisms. It has been shown to be very toxic to guppies (Hopf *et al.*, 1967) and other fish (Johnson & Finley, 1980; Schaefer *et al.*, 1981; Mayer & Ellersieck, 1986). Information concerning its effects on organisms lower in the food chain, such as cladocerans, is scarce.

Cladocerans are used extensively to determine the toxicity of aquatic pollutants. Various species of cladocerans have been shown to be the predominant organism in diets of some fish (Guma'a, 1978; Hansen & Wahl, 1981). Ease of culture, sensitivity to pollutants, an extensive data base, and a short life cycle in addition to their ecological importance make cladocerans ideal test organisms for this study.

Of the many species of cladocerans used in toxicity testing, *Daphnia magna* are used most frequently. This is due, in part, to the ease in culturing, counting, and handling them. This species is widely used despite its near absence in North American waters (Mount & Norberg, 1984). *Daphnia pulex* and *Ceriodaphnia dubia* both exhibit the desirable quality of large brood size but are much smaller than *D. magna* and, therefore, more difficult to count. In addition, *D. pulex* had a greater tendency to get caught in the water surface film than *D. magna* and *C. dubia* under our test conditions.

The objectives of this study were to determine the acute toxicity of TPTH to the three daphnid species, and compare relative sensitivity of these three species to a pollutant.

MATERIALS AND METHODS

Culturing

Cladocerans were cultured in 1 litre glass beakers containing 900 ml of ultraviolet-sterilized Lake Superior water which had been passed through sand, then through 50 μm fibre and charcoal filters. A temperature range of 20–25°C and a 16 h photoperiod were maintained. Cultures were started one month prior to testing using ten individuals of each species from laboratory cultures (ERL-Duluth). For the purpose of increasing genetic variability, no effort was made to maintain pure strains. *D. magna* and *D. pulex* were fed 1 ml of 5 mg ml^{-1} blended trout food granules and 3 ml algae (*Selenastrum* sp.) media every second day. *C. dubia* received 3 ml of *Selenastrum* sp. media daily along with 3 ml of a yeast–Cerophyl (Agri-Tech) trout food preparation (US EPA, 1985). Culture water was changed twice weekly and beakers were cleaned weekly. Three generations were maintained on a rotational basis at densities of approximately 25 animals per beaker.

Cultures were placed in the test water bath one week prior to testing,

young were removed daily, and the final feeding was performed 24 h prior to the test start.

Test conditions

Each of the five concentrations of TPTH (100 ml) were mixed using a stock solution from a saturator (Veith & Comstock, 1975) and water from the same source as the culture and control water. The mixtures and control water (25 ml) were then added to 30 ml test beakers. Tests were run in triplicate resulting in 18 test beakers for each test species. Range finding tests were performed for each species. Actual test solutions were mixed from a measured concentration stock solution using a 50% dilution factor. Mean test water temperature was $23{\cdot}2 \pm 0{\cdot}25°C$.

Dissolved oxygen, EDTA hardness, total alkalinity, and pH were measured at the beginning and end of each test. Dissolved oxygen ranged from 7·7 to 8·1 mg litre^{-1} and mean total hardness and alkalinity were 46·5 and 40·3 mg litre^{-1} as $CaCO_3$, respectively (APHA, 1980). The range in pH was 7·1 to 7·8.

Testing procedure

A total of three transfers of test organisms were made prior to test initiation. Cladoceran neonates (< 24-h old) were transferred from cultures to clean lake water using a 8 mm glass tube and rubber bulb. After 5 min 90% of the water was removed and replaced with clean lake water. Five minutes later approximately 20 neonates were transferred in a random fashion to each concentration of test and control water for a final 5 min rinse before introducing five animals to each 30 ml test beaker for 48 h.

Each test beaker was observed visually for 2 to 3 min at 24 and 48 h. Any animal showing no movement during this period was considered affected. Animals which were clearly dead were removed. Cladocerans which had become trapped in the surface film were gently resubmerged at 24 h.

Animal handling during the rinsing procedure prior to introducing the animals to the test water was minimized to avoid unnecessary stress to the organisms. The transfer to excess test water instead of more rinse water (APHA, 1985) was done to decrease dilution of the actual test water during introduction of the test animals. This was deemed important due to the small volumes of test water that were being used.

EC_{50} (immobility) values and 95% confidence intervals were calculated using the pooled mortality data from the three replicates for each species and the trimmed Spearman–Karber method (Hamilton *et al.*, 1977). Individual EC_{50}s were calculated for each of three replicates for each test species for the

purpose of performing a one-way analysis of variance to compare the EC_{50} for each species.

Chemical analysis

Proportional dilutions of the measured stock solution were used to determine test beaker concentrations. The TPTH water solution in the saturator reservoir was analysed prior to each bioassay. A 5 ml sampl was transferred to a volumetric flask and simultaneously derivatized to triphenyltin hydride and extracted by adding 5 ml of hexane followed by 2 ml of 4% (w/v) aqueous sodium borohydride (Matthias *et al.*, 1986) and shaken by hand for 3 min. Spiked samples were prepared in deionized water and extracted as stated above.

Standards were prepared by derivatizing and extracting aqueous solutions of TPTH (99·75%, US Environmental Protection Agency, Research Triangle Park, NC). Final working standards were in hexane for gas chromatographic quantification. Analysis was accomplished using a Hewlett Packard Model 5880A gas chromatograph with a model 19305B flame photometric detector (FPD). The FPD was operated in the phosphorus mode with a selective quartz filter for tin emission (600 nm, Ditric Optics, Hudson, MA). A 180 cm × 2 mm ID glass column packed with 3% OV-1 on 80/100 mesh W-HP was used with nitrogen carrier gas at a flow rate of 33 ml min^{-1}. The retention time of triphenyltin hydride was 2·7 min. All concentrations are reported as triphenyltin hydroxide and the mean recovery from spiked water samples was $95·6 \pm 8·2\%$ ($n = 10$).

RESULTS AND DISCUSSION

All cladocerans survived at the lowest TPTH concentrations which varied no more than 0·6 μg $litre^{-1}$ (Table 1). High concentrations ranged from 39·1 to 49·0 μg $litre^{-1}$. There was one surviving *C. dubia* in the high concentration, 42·9 μg $litre^{-1}$, although all died at 21·4 μg $litre^{-1}$. At TPTH concentrations of 9·8 μg $litre^{-1}$ to 12·2 μg $litre^{-1}$, *D. pulex* and *D. magna* were not acutely affected (immobilized) although *C. dubia* was slightly affected in this range. Effects were exhibited in all species at TPTH concentrations of 20 μg $litre^{-1}$ and above.

All three species are of the Daphnidae family. Similarity of tolerance to a toxin within a family is common. The greatest difference in EC_{50}s is that of *D. magna* being greater than *C. dubia* by 1·5 times (Table 2). Analysis of variance using EC_{50}s for each replicate revealed no significant difference ($P > 0·05$) in sensitivity to TPTH among the species. This lack of significance

TABLE 1
Percentage Survival of Cladocerans after 48-h Exposure to Triphenyltin Hydroxide (Values are mean ± SE of the three replicates)

Daphnia pulex		Daphnia magna		Ceriodaphnia dubia	
Concentration ($\mu g\ litre^{-1}$)	*Survival* (%)	*Concentration* ($\mu g\ litre^{-1}$)	*Survival* (%)	*Concentration* ($\mu g\ litre^{-1}$)	*Survival* (%)
0	100 ± 0	0	86·7 ± 10·3	0	100 ± 0
2·5	100 ± 0	3·1	100 ± 0	2·7	100 ± 0
4·9	93·3 ± 5·1	6·2	93·3 ± 5·1	5·3	93·3 ± 5·1
9·8	93·3 ± 5·1	12·2	86·7 ± 5·1	10·8	60·0 ± 0
19·6	20·0 ± 15·5	24·6	13·3 ± 5·1	21·4	0 ± 0
39·1	0 ± 0	49·0	0 ± 0	42·9	6·7 ± 5·1

is supported by the overlap of 95% confidence intervals for all species (Table 2).

While no data were found concerning the effects of TPTH on cladocerans, Johnson & Finley (1980) and Mayer & Ellersieck (1986) reported a 96-h LC_{50} of 66 $\mu g\ litre^{-1}$ with *Gammarus fasciatus*. Tributyltin oxide (TBTO), another triorganotin compound, was shown to have a 48-h EC_{50} of 4·3 $\mu g\ litre^{-1}$ with *D. magna* (Brooke *et al.*, 1986). TBTO appeared to be more toxic than TPTH when compared to the 48-h EC_{50} of 16·5 $\mu g\ litre^{-1}$ for *D. magna* in our study.

Fish also appear to be quite sensitive to TPTH. Jarvinen *et al.* (unpublished data) found fathead minnow (*Pimephales promelas*) fry to have a 96-h LC_{50} of 7·1 $\mu g\ litre^{-1}$. Rainbow trout (*Salmo gairdneri*) fry and bluegill (*Lepomis macrochirus*) fry had 96-h LC_{50}s of $< 28\ \mu g\ litre^{-1}$ and 23 $\mu g\ litre^{-1}$, respectively (Johnson & Finley, 1980; Mayer & Ellersieck, 1986).

The narrow range of TPTH concentrations causing acute toxicity (a 48-h

TABLE 2
EC_{50}s (From Pooled Replicates) for Cladocerans Exposed to Triphenyltin Hydroxide and Corresponding 95% Confidence Intervals

Species	*48-h* EC_{50} ($\mu g\ litre^{-1}$)	*95% CI* ($\mu g\ litre^{-1}$)
Ceriodaphnia dubia	11·3	9·2–14·0
Daphnia pulex	14·5	12·0–17·5
Daphnia magna	16·5	13·6–20·1

EC_{50} of 11·3 μg litre^{-1} for *C. dubia* (this study) to a 96-h LC_{50} of 66 μg litre^{-1} for *Gammarus fasciatus* (Johnson & Finley, 1980; Mayer & Ellersieck, 1986)), indicates very little selectivity for species. All of the values show this compound to be highly toxic. Hall & Pinkney (1985) found organotin compounds, in general, to be very toxic to aquatic biota.

Jarvinen *et al.* (unpublished data) found effects of TPTH to be cumulative with fathead minnow (*Pimephales promelas*) fry. Prolonged or repeated exposure may, therefore, cause TPTH to be more toxic in chronic tests than would be expected based on results of acute tests.

The sensitivity to TPTH exhibited by cladocerans indicates other invertebrates may be sensitive. The toxicity to these fish forage items, in addition to the primary toxicity to fish, indicates that great care needs to be taken when applying this compound in or around aquatic environments.

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